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Brain region specific modulation of ethanol-induced depression of GABAergic neurons in the brain reward system by the nicotine receptor antagonist mecamylamine☆

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A B S T R A C T

The mechanisms underlying ethanol-induced activation of the mesolimbic dopamine system are not fully understood, but increased extracellular dopamine in the nucleus accumbens (nAc) has been shown to involve nicotinic acetylcholine receptors (nAChRs). Basal activity of dopaminergic neurons in the ventral tegmental area (VTA) is under the influence of GABAergic neurotransmission, and the aim of this study was to characterize the involvement of nAChRs in mediating acute ethanol effects on GABAergic activity in subregions of the brain reward system. Multi-electrode *in vivo* recordings were made in the VTA and nAc of awake and behaving C57BL6/J mice receiving intraperitoneal injections of saline or ethanol (2.0 g/kg), combined with, or without, pre-injection of the non-competitive nAChR antagonist mecamylamine (1.0 mg/kg). Ethanol significantly decreased the activity of quinpirole-insensitive slow-spiking and fast-spiking units in both the VTA and the nAc as compared to saline injection. Pre-treatment with mecamylamine inhibited the rate-inhibiting properties of ethanol in the VTA, but not in the nAc. The data presented here show that ethanol depresses the activity of quinpirole-insensitive, putative GABAergic neurons, in the mesolimbic dopamine system of mice, and that nAChRs contribute to this modulation. This finding, taken together with previous microdialysis studies, supports an involvement of GABAergic neurons and nAChRs in ethanol's interaction with the mesolimbic dopamine system.

Introduction

The nucleus accumbens (nAc) and its reciprocal connection with the ventral tegmental area (VTA) are primary components of the mesolimbic dopamine system, which is activated by drugs of abuse, including ethanol (Di Chiara & Imperato, 1986). Ethanol administration elevates extracellular dopamine in the nAc, and pharmacological manipulation of neuronal activity within the nAc or afferent circuit systems modulates ethanol self-administration (Blomqvist, Ericson, Johnson, Engel, & Söderpalm, 1996; Chau, Höifödt-Lidö, Löf, Söderpalm, & Ericson, 2010; Ericson, Blomqvist, Engel, & Söderpalm, 1998; Hyytiä & Koob, 1995). Even though ethanol has been shown to increase the firing rate of DA neurons both *in vivo* and *in vitro* (Brodie, Shefner, & Dunwiddie, 1990; Gessa, Muntoni, Collu, Vargiu, & Mereu, 1985), it has been suggested that their excitation may be attributed to disinhibition connected with reduced GABAergic neurotransmission (Mereu & Gessa, 1985; Theile, Morikawa, Gonzales, & Morrisett, 2011). In fact, GABAergic neurotransmission has repeatedly been shown to regulate the activity of dopaminergic neurons in the VTA (Jhou, Fields, Baxter, Saper, & Holland, 2009; Kalivas, 1993; Walaas & Fonnum, 1980). Stimulation of GABAergic interneurons in the VTA leads to a decrease in the downstream release of dopamine in the nAc, and activation of VTA $GABA_A$ receptors reduces ethanol intake in mice (Moore & Boehm, 2009). Furthermore, the $GABA_A$ receptor inhibitor bicuculline is self-administered in the VTA (David, Durkin, & Cazala, 1997), suggesting that changes in GABAergic transmission in brain regions associated with the mesolimbic dopamine system could be important regulators of reward (van Zessen, Phillips, Budygin, & Stuber, 2012).

Previous research has shown that the increase in accumbal dopamine levels induced by intraperitoneal (i.p.) injection of ethanol requires activation of nicotinic acetylcholine receptors (nAChRs) (Blomqvist, Engel, Nissbrandt, & Söderpalm, 1993; Larsson, Svensson, Söderpalm, & Engel, 2002). The nAChR antagonist mecamylamine

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also reduces ethanol self-administration in rodents (Blomqvist et al., 1996; Ericson et al., 1998; Hendrickson, Zhao-Shea, & Tapper, 2009), while antagonists selective for α4β2 or homomeric α7 have no significant effect on consumption (Hendrickson et al., 2009; Lê, Corrigall, Harding, Juzytsch, & Li, 2000). Further supporting a role for nAChRs in the reinforcing effects of ethanol are studies showing that ethanol-associated cues promote drug-seeking behavior via activation of nAChRs in the VTA (Löf et al., 2007). Interestingly, GABA neurons projecting from the VTA inhibit accumbal cholinergic interneuron-mediated enhancement of stimulus-outcome learning (Brown et al., 2012), and GABAergic circuits were recently shown to modulate the reinforcement-related signals of cholinergic interneurons (English et al., 2012). Considering the role of GABAergic neurons in regulating dopamine transmission, the aim of this study was to define the involvement of nAChRs in mediating ethanol-induced changes in activity of quinpirole-insensitive neurons in the nAc and VTA of awake and behaving mice. Our data show that ethanol depresses firing frequency, and that mecamylamine inhibits the effect by ethanol in a brain region specific manner.

Material and methods

Animals

All procedures were reviewed and performed in accordance with the Instituto Gulbenkian de Ciência Ethics Committee guidelines, and approved by the Portuguese Veterinary General Board (Direcção Geral de Veterinária, approval ID 018831). Subjects were 4 experimentally naïve adult (3–4 months) male C57BL6/J mice (Jackson). To minimize the number of animals used and allow comparisons in the same individual, all subjects received all the treatments using a modified Latin square design, which controls for order and carry-over effects. Subjects were given at least 24 h between sessions to allow for recovery from injections. Animals were housed under a 12-h dark–light cycle (lights off at 1900). Experimental procedures were performed during the light phase of their cycle. Animals had free access to food and water at all times except during recording sessions.

Surgery

Animals were anesthetized with isoflurane and placed in a stereotaxic apparatus (Kopf Instruments, CA, USA). The scalp was shaved and swabbed with iodine. A central incision was made to expose the skull and two craniotomies approximately 1.5 mm wide and 1.5 mm long were drilled in the skull's left side (−3.0 mm AP, +0.75 mm ML, −4.2 mm DV for VTA; +1.45 mm AP, +0.875 mm ML, −4.25 mm DV for nAc; all coordinates relative to bregma). A 16-microelectrode array (CD Neural Technologies; Durham, NC, USA) configured in four rows of four microelectrodes, with 200 μm of space between electrodes within a row, and 200 μm between rows was lowered into each target brain region. Neural activity was monitored online while lowering electrodes into the target nuclei to ensure proper electrode depth and positioning. Ground wires were wrapped around skull screws, and the microelectrode arrays were anchored with dental acrylic (Lang Dental, IL, USA), using the skull screws as anchors. Subjects were given at least 5 days to recover after surgery before beginning experimental procedures.

In vivo recordings

On the day of the recording, animals' microelectrodes were connected to a printed circuit board (PCB) linked to a recording preamplifier (Costa, Cohen, & Nicolelis, 2004), and neuronal activity was recorded using the MAP system (Plexon, Inc., TX, USA) and the Cerebus data acquisition system (Blackrock Microsystems, UT, USA) (Costa et al., 2006). Baseline activity was recorded from subjects for 10 min, after which the nAChR antagonist mecamylamine (2-(methylamino)isocamphane hydrochloride, N,2,3,3 tetramethylbicyclo[2.2.1]heptan-2-amine hydrochloride; Sigma-Aldrich, Saint Louis, MO, USA) was injected (1.0 mg/kg, 0.01 mL/g). Post-mecamylamine data were recorded for an additional 20 min, after which subjects received either saline or ethanol injection (2.0 g/kg from a 30% solution). Post-treatment recording lasted an additional 20 min. To separate dopaminergic neurons from GABAergic neurons, mice were injected with the D_2R agonist quinpirole ((−) quinpirole monohydrochloride, trans-(−)-(4aR)-4,4a,5,6,7,8,8a,9-octahydro-5-propyl-1H-pyrazolo[3,4-g]quinolone monohydrochloride; Sigma-Aldrich) at the end of the experiment (3.0 mg/kg, 0.01 mL/g). All drugs were dissolved in 0.9% saline, which also served as the control injection.

Data analysis

Subsequent to recording, units were further sorted and clarified offline via Offline Sorter (Plexon, Inc., TX, USA). Tracking principle components continuously over time verified that the same units were recorded over the entire course of one experimental session. If a unit was lost at any point in a session, the unit was excluded from analysis. Across all sessions, an average of 18.4 ± 1.2 total units was recorded per subject, with 13.1 ± 0.8 from the VTA and 5.4 ± 0.6 from the nAc.

Even though extracellular recordings do not permit definitive classification of neurons, consensus indicates that careful application of established criteria in conjunction with attention to electrode localization is valid and sufficient to identify neuronal subtypes. Assessed parameters for classifying GABAergic neurons were based on clustering of established *in vivo* electrophysiological characteristics including firing rate during baseline recording (before drug injection), amplitude of the spikes as the maximal peak-valley difference for each neuronal waveform, and half-width of each waveform as the valley width at the half-maximum of spike amplitude, and the lack of pharmacological response to the D_2 agonist quinpirole (Burkhardt, Jin, & Costa, 2009; Jin & Costa, 2010; Steffensen, Svingos, Pickel, & Henriksen, 1998; Ungless & Grace, 2012; Yin et al., 2009) (Fig. 1A). The different categories showed distinct clustering across assessed electrophysiological parameters (Table 1).

Average firing rate frequency was calculated in 50-s bins for 600 s immediately before and after the injection time, and pre-injection bins were compared against post-injection bins using single-factor ANOVA ($\alpha = 0.05$). If this comparison proved significant, the neuron was considered to show a change in activity. Population rate vectors were generated using the average firing rate for each neuron in 10-s bins.

Localization of recording electrodes

Final localization of recording electrodes was confirmed *post mortem*. Subjects were perfused transcardially with 10% formalin, and intact brains were extracted. Prior to sectioning, brains were cryoprotected overnight in a 25% sucrose solution at 4 °C. Sections were cut at a 30-μm thickness on a sliding microtome, mounted on gelatin-subbed slides, and coverslips were sealed with nitrocellulose adhesive. Tracks from microelectrodes were confirmed visually under a light microscope (Fig. 1B–D).

Statistical analysis

Statistical comparisons were performed using the SPSS (SPSS, Chicago, IL, USA) software package. Changes in firing rate in

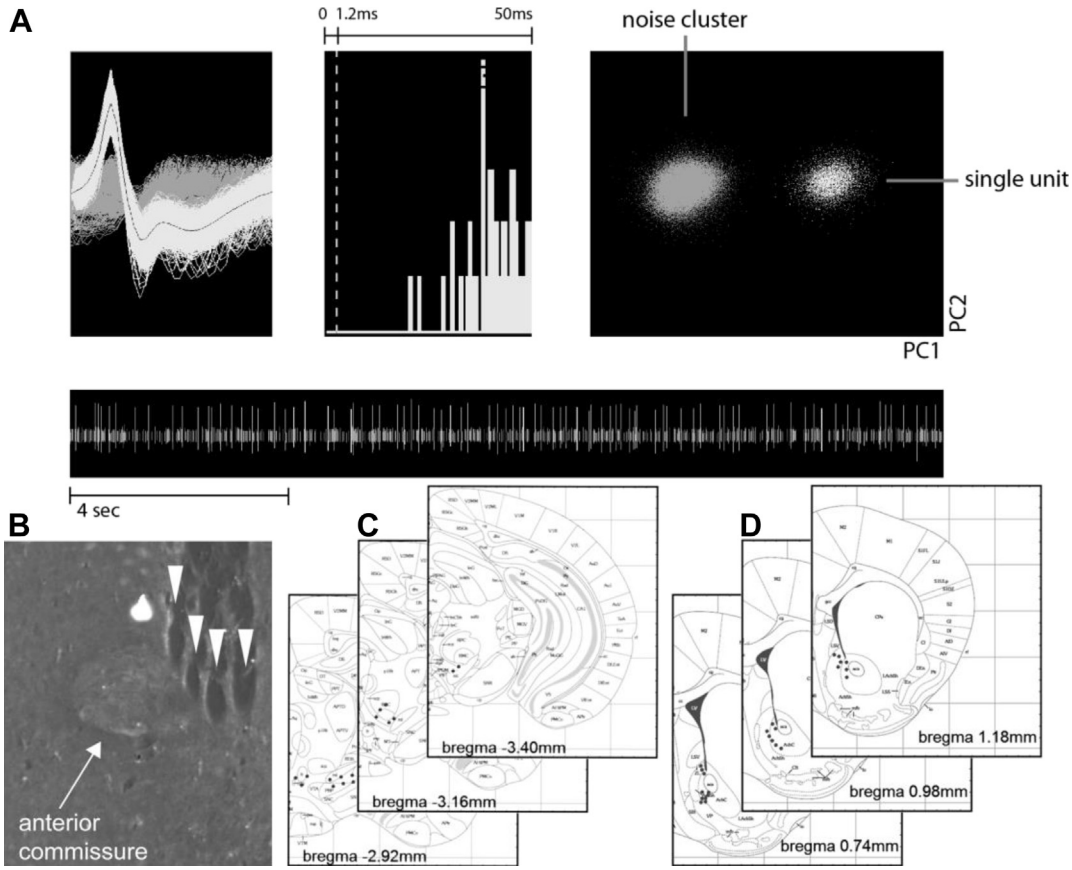
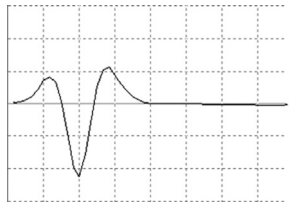
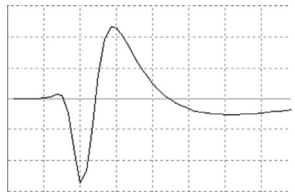
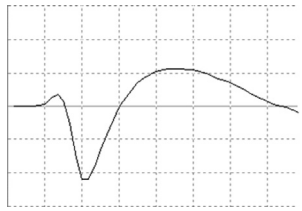


Fig. 1. Waveform analysis and histology. A) Example of sorted single unit, recorded one week subsequent to electrode implantation. From left: unit waveforms (dark gray) isolated from noise (light gray) (*x*-axis, 1200 μs; *y*-axis, 500 μV); interspike interval histogram; isolated unit and noise on a principal component plot (*x*-axis, PC1; *y*-axis, PC2); bottom, raster trace of unit events and noise events over a selected time period. B) Photomicrograph of representative section in nAc, illustrating microelectrode tracks and tip location. C, D) Dots indicate observed terminus of a microelectrode track within the tissue in VTA (C) and nAc (D).

Table 1
Waveform properties of recorded units.

Unit	Peak valley time (μs)	Amplitude ratio	Frequency (Hz)	Waveform ($x = 200$ μs, $y = 71$ μV)
VTA GABAergic slow-spiking	161 ± 5.6	1.47 ± 0.02	11 ± 0.66	
VTA GABAergic fast-spiking	167 ± 7.3	1.33 ± 0.03	35 ± 2.0	
nAc GABAergic slow-spiking	348 ± 12.8	1.60 ± 0.03	3.11 ± 0.55	

individual neurons were analyzed using single-factor ANOVA. Data describing relative amount of neurons responding with changes in firing rate were based on four separate sessions using four different mice unless anything else is clearly stated, and presented as mean values with standard deviation. The group effect, where the total number of neurons showing a change in rate following administration of ethanol was compared to saline treatment, was defined using Fisher's exact test. Normalized population vectors were compared over time using 2-way ANOVA. All graphs were assembled in GraphPad Prism (GraphPad Software, Inc., San Diego, CA).

Results

Role of nAChRs in regulating ethanol-induced changes in firing rate

Quinpirole-insensitive neurons were categorized based on distinct clustering across assessed electrophysiological parameters, into VTA slow-spiking, VTA fast-spiking, and nAc slow-spiking GABAergic neurons (Table 1). Activity of individual neurons was compared 600 s before and 600 s after a systemic injection of either saline or ethanol (2.0 g/kg) using paired *t* test, and sorted into three possible outcomes: increased, decreased, and unaltered firing rate. In the VTA, the absolute number of GABAergic slow-spiking neurons showing no change in firing rate was significantly reduced in response to ethanol administration (Fisher's exact test: $p = 0.026$, saline $n = 45$, ethanol $n = 38$) (Fig. 2A), and there was a significant increase in the percentage of neurons responding with decreased firing rate (Fisher's exact test: $p < 0.001$) (Fig. 2A). There was a similar, although not significant, trend toward depressed firing rate in VTA fast-spiking neurons in response to ethanol administration (Fisher's exact test: $p = 0.075$; saline $n = 13$, ethanol $n = 21$) (Fig. 2D). The firing rate of quinpirole-insensitive slow-spiking GABAergic neurons in the nAc was robustly depressed by ethanol administration (Fisher's exact test: $p < 0.001$, saline $n = 19$, 3 sessions, ethanol $n = 23$), and there was a decline in the number of unaffected neurons as compared to saline-injection ($p = 0.001$) (Fig. 2G).

Pre-treatment with the non-competitive nAChR antagonist mecamylamine (1.0 mg/kg) blocked the rate-depressing effect of ethanol on GABAergic neurons in the VTA (slow-spiking neurons: Fisher's exact test: $p = 0.59$, saline $n = 41$, ethanol $n = 27$; fast-spiking: $p = 0.59$, saline $n = 12$, ethanol, $n = 13$, 3 sessions) (Fig. 2B, E). In slow-spiking units in the nAc, however, the rate depressant effect displayed by ethanol remained (Fisher's exact test: $p = 0.002$, saline $n = 16$, ethanol $n = 21$) (Fig. 2H).

Ethanol-induced changes in normalized population vectors

In an attempt to further determine the overall impact on ethanol-induced changes in firing rate, normalized population vectors were

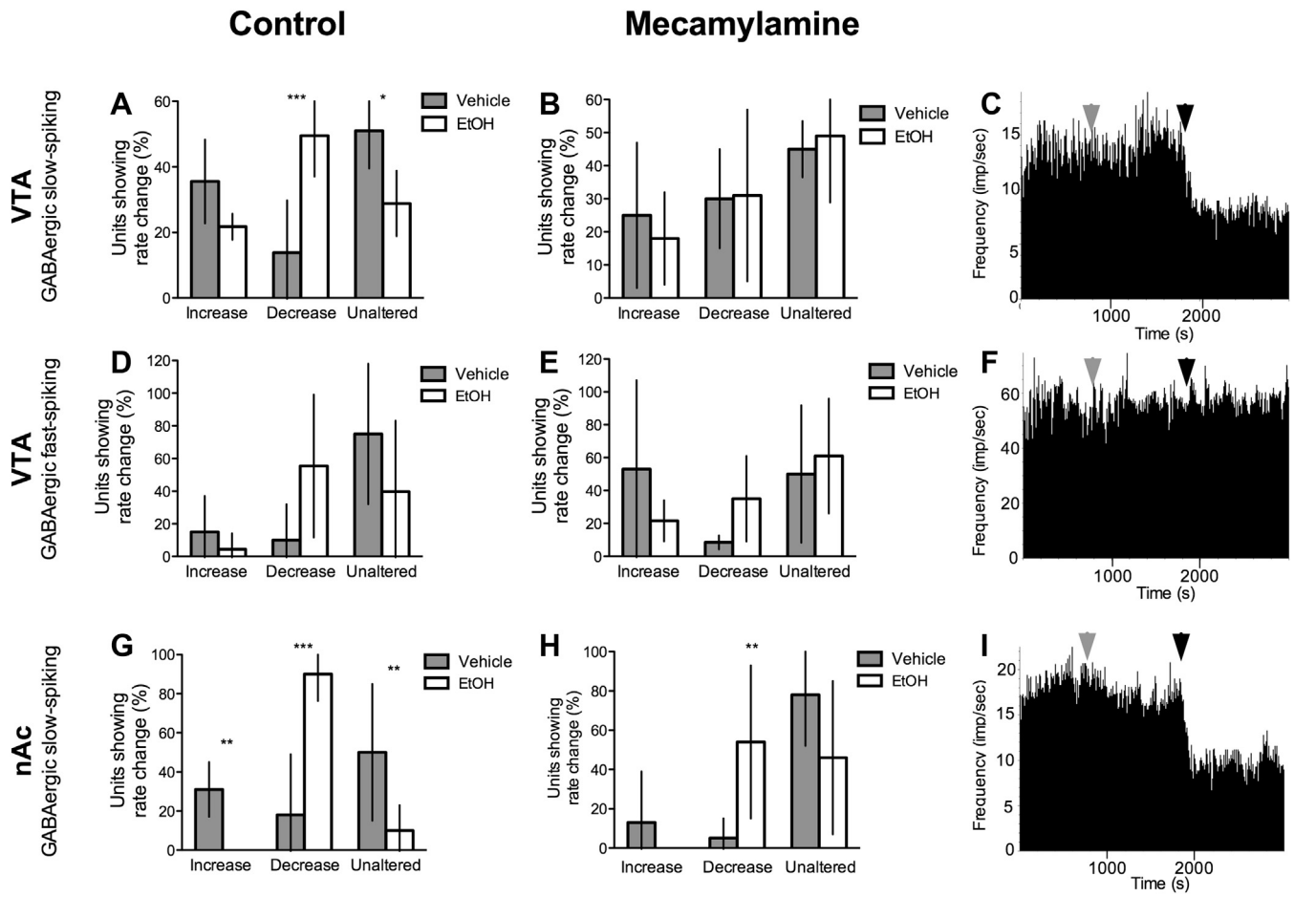


Fig. 2. Ethanol-induced changes in firing rate are regulated by nAChRs in a subregion specific manner. Bar graphs show percentage of neurons changing firing frequency, with SD based on 3–4 sessions. Ethanol administration (2.0 g/kg) depressed firing rate in slow-spiking GABAergic neurons in the VTA (A) and the nAc (G), while VTA fast-spiking units were not significantly affected as compared to saline injection (D). Pre-treatment with the non-competitive nAChR antagonist mecamylamine (1.0 mg/kg) prevented the decrease in firing rate induced by ethanol in GABAergic neurons in the VTA (B), but not in the nAc (H). Example traces showing firing frequency over time in VTA slow-spiking (C), VTA GABAergic fast-spiking (F) and nAc slow-spiking units (I). Arrows mark time points for administration of mecamylamine (gray) and ethanol (black). *< 0.05, **< 0.01, ***< 0.001, significant compared to saline treatment.

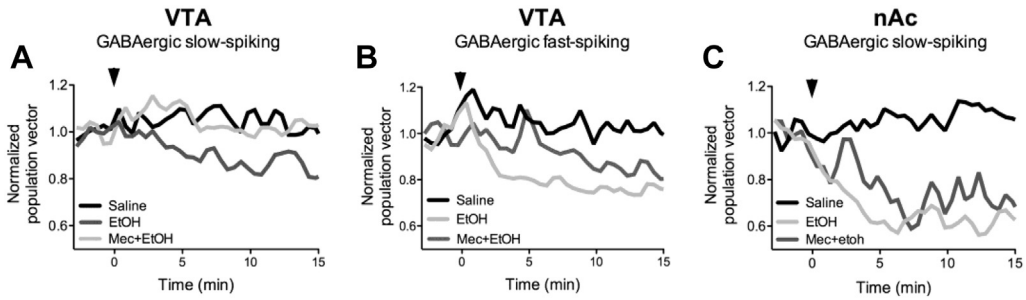


Fig. 3. Normalized population vectors describing collective activity over time. Ethanol administration (2.0 g/kg) significantly depressed normalized population vectors as compared to vehicle-treated control in both the VTA and nAc. In the VTA (A–B), normalized population vectors from mice receiving ethanol after mecamylamine pretreatment did not deviate significantly from vehicle-treated controls, while the robust depression sustained in the nAc (C). EtOH; ethanol. Mec; mecamylamine.

generated and compared to saline-treated controls. In the VTA, the normalized population vector of GABAergic slow-spiking neurons deviated significantly from saline-treated controls (2-way ANOVA, $F = 10.5$, $p < 0.01$), while mecamylamine-pretreated neurons receiving ethanol did not (2-way ANOVA, $F = 2.13$, $p > 0.05$) (Fig. 3A). Ethanol also depressed the normalized population vector of quinpirole-insensitive fast-spiking VTA neurons (2-way ANOVA, $F = 10.5$, $p < 0.001$), but not in mice pre-treated with mecamylamine (2-way ANOVA, $F = 2.09$, $p > 0.05$) (Fig. 3B). In the nAc, normalized population vectors were robustly depressed by ethanol in both control (2-way ANOVA, $F = 45$, $p < 0.001$), and mecamylamine-pre-treated mice (2-way ANOVA, $F = 20$, $p < 0.001$) (Fig. 3C).

Effects on neuronal activity by mecamylamine administration

Mecamylamine administration did not significantly modulate neuronal firing rate in any direction in individual neurons, and there was no change in normalized population rate over time (Fig. 4).

Considering the risk of physiological effects caused by the repeated-measures design employed in this study, particular attention was paid to baseline comparisons across different conditions. Mean firing frequency for categorized neurons in different brain regions and mice was not significantly altered across sessions (Fig. 5). This suggests that long-term effects on synaptic activity, or damage of neurons and supporting structures induced by ethanol or mecamylamine, were not a factor in the observed results, and that neuronal activity in the VTA and nAc is robust over time in this experimental design.

Discussion

The data presented here show that systemic ethanol administration depresses the activity of quinpirole-insensitive neurons in the VTA and nAc of awake and freely moving mice. We also show, for the first time, that nAChRs are involved in regulating this effect in the VTA, but not in the nAc. This brain region specific finding agrees with previous reports from rat studies, showing that enhanced accumbal dopamine release induced by ethanol is inhibited by mecamylamine administered in the VTA, but not in the nAc (Blomqvist et al., 1993; Blomqvist, Ericson, Engel, & Söderpalm, 1997; Ericson et al., 1998).

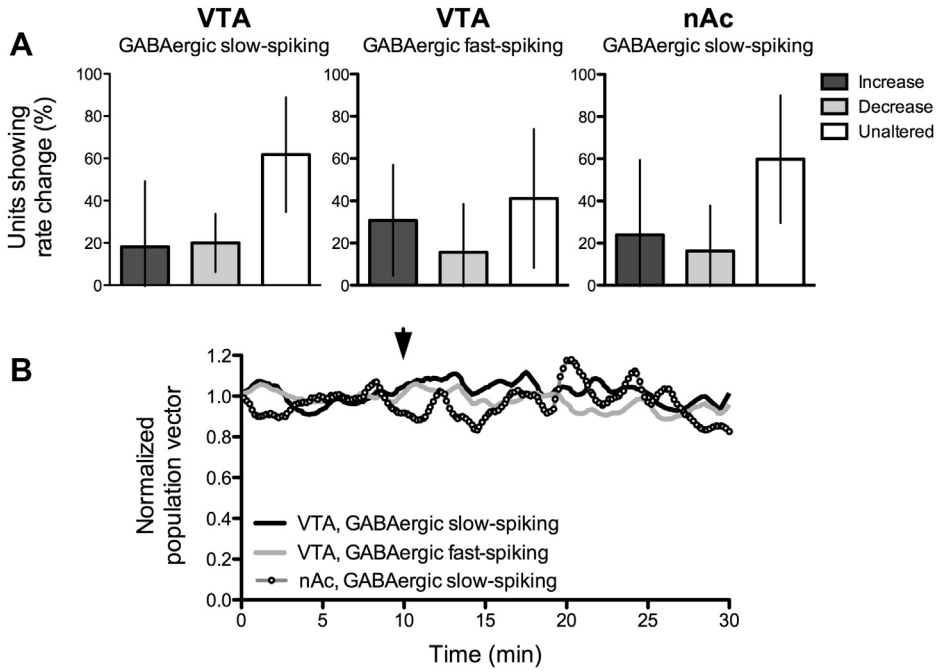


Fig. 4. Mecamylamine does not modulate GABAergic firing frequency. A) Bar graphs show relative number of neurons responding with a change in firing frequency with SD based on 3–4 sessions. Firing rate was not significantly shifted toward increased or decreased firing rate in response to mecamylamine administration (1.0 g/kg) in either brain region. B) Normalized population rate of GABAergic neurons in the VTA and nAc, presented over time in 10-s bins. Arrow marks time point for mecamylamine administration.

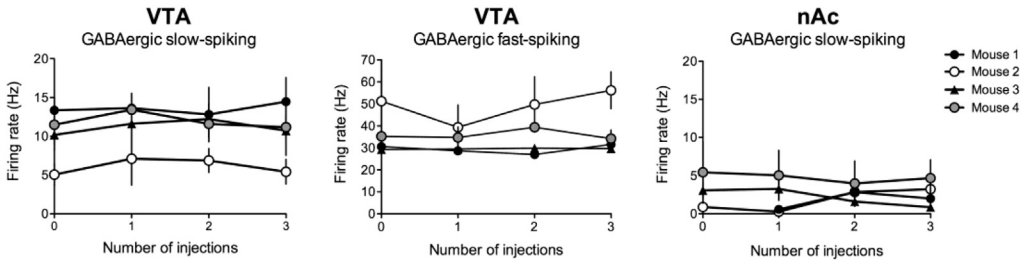


Fig. 5. Baseline firing frequency is not modulated across sessions. Baseline firing rate was not significantly altered across sessions, suggesting that neuronal activity in the VTA and nAc is robust over time in this experimental design and that long-term effects on synaptic activity are not a factor in the observed results.

The neurons presented in this study were categorized as GABAergic or dopaminergic based on electrophysiological properties and pharmacological response to quinpirole (Table 1). Even though a subpopulation of dopaminergic neurons do not possess D_2 autoreceptors and thus do not show inhibition in response to quinpirole, these are primarily localized in the medial aspect of the VTA (Chiodo, Bannon, Grace, Roth, & Bunney, 1984). The data presented here did not include any neurons that exhibited the standard electrophysiological parameters of dopaminergic neurons (Ungless, Magill, & Bolam, 2004).

Ethanol exerts a complex effect on neuronal circuitry by interacting with multiple receptor systems and by both activating and inhibiting the function of proteins involved in synaptic transmission (Lovinger & Roberto, 2013; Vengeliene, Bilbao, Molander, & Spanagel, 2008). Interestingly, the data presented here suggest that ethanol depresses the activity of GABAergic neurons in both the VTA and the nAc, but that the neurobiological underpinnings are brain region specific. Subregion specific effects by ethanol have previously been shown in the VTA, where the opioidergic tone appears to be an important regulator in determining whether ethanol has a net depressant or activating effect on sIPSCs and neuronal firing (Guan et al., 2012). Considering the possible involvement of opioid receptors in the behavioral effects of nicotine, and the influence by nicotine on opioidergic function, it is thus possible that there is a direct interaction between these systems in the VTA (Berrendero et al., 2012; Hadjiconstantinou & Neff, 2011). However, the nAChR involvement could also be due to a direct interaction between ethanol and nAChRs or to an indirect modulation due to ethanol-induced acetylcholine release. In fact, concomitant with the elevation of dopamine in the nAc, voluntary ethanol intake in ethanol-preferring rats has been shown to increase extracellular acetylcholine levels in the VTA (Larsson, Edström, Svensson, Söderpalm, & Engel, 2005). Furthermore, ethanol intake and preference, as well as dopamine release in the nAc, correlate with VTA acetylcholine levels, supporting a close association between ethanol and acetylcholine in the VTA (Larsson et al., 2005). The data presented here show that inhibition of nAChRs by systemic injection of mecamylamine leaves neuronal activity relatively unaffected. This is also congruent with the lack of effect of mecamylamine on basal dopamine release in the nAc (Blomqvist et al., 1997), indicating a low basal acetylcholine tone.

In the nAc, the acute effect displayed by ethanol on neurotransmission appears to involve glycine receptors, and this interaction might entail nAChR involvement (Adermark et al., 2011; Ericson, Molander, Löf, Engel, & Söderpalm, 2003; Söderpalm, Löf, & Ericson, 2009). However, elevated dopamine levels as a result of local administration of ethanol or taurine in the nAc are also inhibited by blockade of VTA nAChRs (Ericson et al., 2003; Ericson, Molander, Stomberg, & Söderpalm, 2006). It has thus been proposed that reduced activity of MSNs, as a result of ethanol administration, leads to enhanced accumbal dopamine output through disinhibition of cholinergic projections terminating in the VTA (Söderpalm et al., 2009). Based on this hypothesis, the reduced firing rate of MSNs could potentially lead to disinhibition of VTA GABA neurons, which might counterbalance the acute depressant effect displayed by ethanol. Concomitant disinhibition of VTA GABAergic neurons could thus explain the relatively smaller effect on population activity in the VTA as compared to the nAc (Fig. 3). Importantly, the depressant effect of ethanol remained in the nAc of mecamylamine-treated mice. Thus, the possible influence on dopamine release caused by local disinhibition of dopaminergic terminals by MSNs does not appear to significantly contribute to ethanol-induced dopamine overflow measured by microdialysis (Söderpalm, Ericson, Olausson, Blomqvist, & Engel, 2000).

In conclusion, the data presented here show that ethanol depresses the firing rate of GABAergic neurons in the VTA and nAc, and that mecamylamine inhibits this influence by selectively affecting GABAergic neurons in the VTA. Such a region-specific blockade by mecamylamine has been reported also with respect to ethanol-induced dopamine elevations in the nAc (Blomqvist et al., 1996), but whether the mecamylamine blockade of ethanol's influence on GABAergic neurons in the VTA is related to the inhibition of the dopamine elevation remains to be determined.

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References

Adermark, L., Clarke, R. B., Olsson, T., Hansson, E., Söderpalm, B., & Ericson, M. (2011). Implications for glycine receptors and astrocytes in ethanol-induced elevation of dopamine levels in the nucleus accumbens. *Addiction Biology, 16*, 43–54.

Berrendero, F., Plaza-Zabala, A., Galeote, L., Flores, Á., Bura, S. A., Kieffer, B. L., et al. (2012). Influence of δ-opioid receptors in the behavioral effects of nicotine. *Neuropsychopharmacology, 37*, 2332–2344.

Blomqvist, O., Engel, J. A., Nissbrandt, H., & Söderpalm, B. (1993). The mesolimbic dopamine-activating properties of ethanol are antagonized by mecamylamine. *European Journal of Pharmacology, 249*, 207–213.

Blomqvist, O., Ericson, M., Engel, J. A., & Söderpalm, B. (1997). Accumbal dopamine overflow after ethanol: localization of the antagonizing effect of mecamylamine. *European Journal of Pharmacology, 334*, 149–156.

Blomqvist, O., Ericson, M., Johnson, D. H., Engel, J. A., & Söderpalm, B. (1996). Voluntary ethanol intake in the rat: effects of nicotinic acetylcholine receptor blockade or subchronic nicotine treatment. *European Journal of Pharmacology, 314*, 257–267.

Brodie, M. S., Shefner, S. A., & Dunwiddie, T. V. (1990). Ethanol increases the firing rate of dopamine neurons of the rat ventral tegmental area in vitro. *Brain Research, 508*, 65–69.
Brown, M. T., Tan, K. R., O'Connor, E. C., Nikonenko, I., Muller, D., & Lüscher, C. (2012). Ventral tegmental area GABA projections pause accumbal cholinergic interneurons to enhance associative learning. *Nature, 492*, 452–456.
Burkhardt, J. M., Jin, X., & Costa, R. M. (2009). Dissociable effects of dopamine on neuronal firing rate and synchrony in the dorsal striatum. *Frontiers in Integrative Neuroscience, 3*, 28.
Chau, P., Höifödt-Lidö, H., Löf, E., Söderpalm, B., & Ericson, M. (2010). Glycine receptors in the nucleus accumbens involved in the ethanol intake-reducing effect of acamprosate. *Alcoholism: Clinical and Experimental Research, 34*, 39–45.
Chiodo, L. A., Bannon, M. J., Grace, A. A., Roth, R. H., & Bunney, B. S. (1984). Evidence for the absence of impulse-regulating somatodendritic and synthesis-modulating nerve terminal autoreceptors on subpopulations of mesocortical dopamine neurons. *Neuroscience, 12*, 1–16.
Costa, R. M., Cohen, D., & Nicolelis, M. A. (2004). Differential corticostriatal plasticity during fast and slow motor skill learning in mice. *Current Biology, 14*, 1124–1134.
Costa, R. M., Lin, S. C., Sotnikova, T. D., Cyr, M., Gainetdinov, R. R., Caron, M. G., et al. (2006). Rapid alterations in corticostriatal ensemble coordination during acute dopamine-dependent motor dysfunction. *Neuron, 52*, 359–369.
David, V., Durkin, T. P., & Cazala, P. (1997). Self-administration of the GABAA antagonist bicuculline into the ventral tegmental area in mice: dependence on D2 dopaminergic mechanisms. *Psychopharmacology, 130*, 85–90.
Di Chiara, G., & Imperato, A. (1986). Preferential stimulation of dopamine release in the nucleus accumbens by opiates, alcohol, and barbiturates: studies with transcerebral dialysis in freely moving rats. *Annals of the New York Academy of Sciences, 473*, 367–381.
English, D. F., Ibanez-Sandoval, O., Stark, E., Tecuapetla, F., Buzsáki, G., Deisseroth, K., et al. (2012). GABAergic circuits mediate the reinforcement-related signals of striatal cholinergic interneurons. *Nature Neuroscience, 15*, 123–130.
Ericson, M., Blomqvist, O., Engel, J. A., & Söderpalm, B. (1998). Voluntary ethanol intake in the rat and the associated accumbal dopamine overflow are blocked by ventral tegmental mecamylamine. *European Journal of Pharmacology, 358*, 189–196.
Ericson, M., Molander, A., Löf, E., Engel, J. A., & Söderpalm, B. (2003). Ethanol elevates accumbal dopamine levels via indirect activation of ventral tegmental nicotinic acetylcholine receptors. *European Journal of Pharmacology, 467*, 85–93.
Ericson, M., Molander, A., Stomberg, R., & Söderpalm, B. (2006). Taurine elevates dopamine levels in the rat nucleus accumbens; antagonism by strychnine. *The European Journal of Neuroscience, 23*, 3225–3229.
Gessa, G. L., Muntoni, F., Collu, M., Vargiu, L., & Mereu, G. (1985). Low doses of ethanol activate dopaminergic neurons in the ventral tegmental area. *Brain Research, 348*, 201–203.
Guan, Y., Xiao, C., Krnjevic, K., Xie, G., Zuo, W., & Ye, J. H. (2012). GABAergic actions mediate opposite ethanol effects on dopaminergic neurons in the anterior and posterior ventral tegmental area. *The Journal of Pharmacology and Experimental Therapeutics, 341*, 33–42.
Hadjiconstantinou, M., & Neff, N. H. (2011). Nicotine and endogenous opioids: neurochemical and pharmacological evidence. *Neuropharmacology, 60*, 1209–1220.
Hendrickson, L. M., Zhao-Shea, R., & Tapper, A. R. (2009). Modulation of ethanol drinking-in-the-dark by mecamylamine and nicotinic acetylcholine receptor agonists in C57BL/6J mice. *Psychopharmacology, 204*, 563–572.
Hyytiä, P., & Koob, G. F. (1995). GABAA receptor antagonism in the extended amygdala decreases ethanol self-administration in rats. *European Journal of Pharmacology, 283*, 151–159.
Jhou, T. C., Fields, H. L., Baxter, M. G., Saper, C. B., & Holland, P. C. (2009). The rostromedial tegmental nucleus (RMTg), a GABAergic afferent to midbrain dopamine neurons, encodes aversive stimuli and inhibits motor responses. *Neuron, 61*, 786–800.
Jin, X., & Costa, R. M. (2010). Start/stop signals emerge in nigrostriatal circuits during sequence learning. *Nature, 466*, 457–462.
Kalivas, P. W. (1993). Neurotransmitter regulation of dopamine neurons in the ventral tegmental area. *Brain Research. Brain Research Reviews, 18*, 75–113.
Larsson, A., Edström, L., Svensson, L., Söderpalm, B., & Engel, J. A. (2005). Voluntary ethanol intake increases extracellular acetylcholine levels in the ventral tegmental area in the rat. *Alcohol and Alcoholism, 40*, 349–358.
Larsson, A., Svensson, L., Söderpalm, B., & Engel, J. A. (2002). Role of different nicotinic acetylcholine receptors in mediating behavioral and neurochemical effects of ethanol in mice. *Alcohol, 28*, 157–167.
Lê, A. D., Corrigall, W. A., Harding, J. W., Juzytsch, W., & Li, T. K. (2000). Involvement of nicotinic receptors in alcohol self-administration. *Alcoholism: Clinical and Experimental Research, 24*, 155–163.
Löf, E., Olausson, P., deBejczy, A., Stomberg, R., McIntosh, J. M., Taylor, J. R., et al. (2007). Nicotinic acetylcholine receptors in the ventral tegmental area mediate the dopamine activating and reinforcing properties of ethanol cues. *Psychopharmacology, 195*, 333–343.
Lovinger, D. M., & Roberto, M. (2013). Synaptic effects induced by alcohol. *Current Topics in Behavioral Neurosciences, 13*, 31–86.
Mereu, G., & Gessa, G. L. (1985). Low doses of ethanol inhibit the firing of neurons in the substantia nigra, pars reticulata: a GABAergic effect? *Brain Research, 360*, 325–330.
Moore, E. M., & Boehm, S. L., 2nd (2009). Site-specific microinjection of baclofen into the anterior ventral tegmental area reduces binge-like ethanol intake in male C57BL/6J mice. *Behavioral Neuroscience, 123*, 555–563.
Söderpalm, B., Ericson, M., Olausson, P., Blomqvist, O., & Engel, J. A. (2000). Nicotinic mechanisms involved in the dopamine activating and reinforcing properties of ethanol. *Behavioural Brain Research, 113*, 85–96.
Söderpalm, B., Löf, E., & Ericson, M. (2009). Mechanistic studies of ethanol's interaction with the mesolimbic dopamine reward system. *Pharmacopsychiatry, 42*(Suppl. 1), S87–S94.
Steffensen, S. C., Svingos, A. L., Pickel, V. M., & Henriksen, S. J. (1998). Electrophysiological characterization of GABAergic neurons in the ventral tegmental area. *The Journal of Neuroscience, 18*, 8003–8015.
Theile, J. W., Morikawa, H., Gonzales, R. A., & Morrisett, R. A. (2011). GABAergic transmission modulates ethanol excitation of ventral tegmental area dopamine neurons. *Neuroscience, 172*, 94–103.
Ungless, M. A., & Grace, A. A. (2012). Are you or aren't you? Challenges associated with physiologically identifying dopamine neurons. *Trends in Neurosciences, 35*, 422–430.
Ungless, M. A., Magill, P. J., & Bolam, J. P. (2004). Uniform inhibition of dopamine neurons in the ventral tegmental area by aversive stimuli. *Science, 303*, 2040–2042.
van Zessen, R., Phillips, J. L., Budygin, E. A., & Stuber, G. D. (2012). Activation of VTA GABA neurons disrupts reward consumption. *Neuron, 73*, 1184–1194.
Vengeliene, V., Bilbao, A., Molander, A., & Spanagel, R. (2008). Neuropharmacology of alcohol addiction. *British Journal of Pharmacology, 154*, 299–315.
Walaas, I., & Fonnum, F. (1980). Biochemical evidence for gamma-aminobutyrate containing fibres from the nucleus accumbens to the substantia nigra and ventral tegmental area in the rat. *Neuroscience, 5*, 63–72.
Yin, H. H., Mulcare, S. P., Hilário, M. R., Clouse, E., Holloway, T., Davis, M. I., et al. (2009). Dynamic reorganization of striatal circuits during the acquisition and consolidation of a skill. *Nature Neuroscience, 12*, 333–341.